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The Phytoremediative Effects of Cilantro (*Coriandrum sativum*) in Lead Contaminated Soil

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Abstract

Environmental exposure to heavy metals such as lead pose a significant health threat. The goal of this project was to evaluate the effectiveness of *Coriandrum sativum* (Cilantro) as a potential lead (II), Pb^{2+}, phytoremediator. *C. sativum* plants were allowed to grow for 43 days while exposed to with different amounts of lead nitrate, $Pb(NO_3)_2$. Lead exposure was accomplished by regular watering with a concentration series of lead nitrate solutions. The roots and shoots of these plants were separated and dried. Ground plant material was liquefied by nitric acid digestion. The amount of lead absorbed by each plant sample was determined by Flame Atomic Absorption Spectrometry (FAAS). A Kruskal-Wallis test found a significant difference between the amounts of lead detected in the plants of the four treatment groups. This difference existed both in the roots and shoots of the *C. sativum*. The results of this study show that greater lead exposure yielded greater lead absorption in *C. sativum*. This supports that *C. sativum* effectively absorbs lead from the environment. Further studies and refined exposure series are needed to increase statistical validity and confirm *C. sativum*'s phytoremediative potential.

Keywords: Coriandrum sativum, lead contamination, bioaccumulation, phytoremediation

Introduction

Heavy metals are prominent contaminants because they do not biodegrade; therefore, they can exist in the soil for thousands of years (Tangahu et al., 2011). They negatively affect plant growth, soil microflora, and other organisms, including humans (Cho-Ruk et al., 2006; Tangahu et al., 2011; Lone et al., 2008). Groundwater can be contaminated and vegetation lost due to lead accumulation (Huang et al., 1997). Lead contamination comes from a variety of everyday products such as car exhaust, paint, explosives, dust, gases from various industrial sources, and chemicals (Tangahu et al., 2011; Sears, 2013; Huang et al., 1997). When present in excess, lead is toxic as it can create oxidative stress by generating free radicals and depleting antioxidant levels in organisms (Flora, Gupta, & Tiwari, 2012). Like other heavy metal contaminants, lead has no metabolic benefit to humans, but instead contributes to acute and chronic diseases when present in excess (Sears, 2013; Lone et al., 2008). Specifically, it has the potential to cause brain damage in humans (Tangahu et al., 2011).

Remediation is used as a way to remove lead from the environment and therefore minimize its toxic effects. While remediation exists in different forms, many are expensive and have unwanted side effects. Remediative approaches can be classified as either physicochemical or biological (Lone et al., 2008). Physicochemical lead remediation includes processes such as incineration, vaporization, solvent washing, and excavation and burial of the soil at a hazardous waste site (Lone et al., 2008; Tangahu et al., 2011) In addition to being costly, these methods can harm the biological component of the soil, disrupt the natural chemical characteristics, and leave behind unnecessary waste (Lone et al., 2008; Tangahu et al., 2011). Alternatively, biological approaches to remediation offer a cheaper and more environmentally friendly alternative to other methods of cleaning pollution (Lone et al., 2008; Cho-Ruk et al., 2006). Biological forms of

remediation include the use of microorganisms and certain plants to decontaminate the environment (Lone et al., 2008). Phytoremediation is an emerging form of bioremediation and can be defined as "the use of plants to clean up a contamination from soils, sediments, and water" (Lone et al., 2008; Tangahu et al., 2011). Many plants have the ability to absorb harmful heavy metals such as lead, cadmium, chromium, arsenic, and various radionuclides from soils (Tangahu et al., 2011). Some plants, accumulators, are especially adept at absorbing heavy metals into their aerial tissues. (Tangahu et al., 2011). They avoid the harmful effects of the contaminants by biodegrading or biotransforming them into inert forms, or by vacuole storage (Tangahu et al., 2011).

Many plants may have the potential for lead phytoremediation that have not yet been tested. If a new plant was found to have remediative properties, it could aid in the removal of lead from the environment and in doing so lessen its negative effects. *C. sativum* has been shown to decrease lead toxicity in the blood because of its chelative properties (Thuppil & Tanner, 2013). If *C. sativum* can chelate lead when fully grown, it is possible that it may also absorb lead from its environment during growth. This preliminary and proof of concept study is designed to examine the phytoremediative properties of *C. sativum* in order to determine whether or not it has the potential to function as a cheap yet effective way to remove lead from the environment. It is hypothesized that there will be a positive correlation between a plant's lead exposure during a defined growth period and the concentration of lead bioaccumulated in the roots and shoots of *C. sativum*, indicating the presence of phytoremediation.

Methods & Materials

Growth of Cilantro and Lead Exposure

Sixteen pots were filled with approximately 1500 ml of Farfard® 3B without Perlite potting soil. Three *C. sativum* seeds were placed in each pot in a triangular formation. 100 ml of soil was added to the top of each pot to cover seeds. Separately, a stock solution was prepared by weighing out 15.98 g of lead nitrate, $Pb(NO_3)_2$ on a scale and adding it to 1 L distilled water. This yielded a concentration of 10,000 mg/L Pb^{2+}. The solution was stirred until completely dissolved. The stock solution was diluted with RO H_2O to produce the desired working concentrations of 50 mg/L, 100 mg/L, 200 mg/L, 1000 mg/L. 1000 ml of the respective aqueous solution was poured into the soil of four pots for each working concentration.

The plants were kept in a climate controlled plant growth chamber at 25°C with 12-hour light cycles. Plants were allowed to grow for 43 days, at which they were fully mature but had not yet begun to wilt. During this growth period, each pot was watered with 100-200 ml of its respective solution derived from the original stock solution one to two times per week, depending on the moisture of the soil. In total, each plant was watered with 1.l L of its respective lead-containing solution.

Preparation of Plant Material

At the end of 43 days, the plants were removed from their pots. The roots and shoots of each plant were rinsed with water to remove any soil particles, and then were cut at the bulge in the stem above ground that clearly divides the roots from the shoots. The plant material was then dehydrated in an oven at over 100°C until completely dry. The dried roots and shoots were separated and stored in resealable plastic bags until ready to be processed. Plant material was

finely ground with a mortar and pestle into a powder. Each sample's plant material was kept separate. Approximately 50 mg from each root sample and 300 mg from each shoot sample was transferred into a 50-ml beaker for acid digestion. Slight variances in masses of plant material were accounted for by dividing the mg/L derived from the standard curve by the mg of tissue processed for that sample. This calculation yielded (mg/L)/mg original plant material.

Acid Digestion and Filtering

One ml of nitric acid, HNO_3, was added to each of the sixteen beakers containing ground roots, and 6 ml were added to those containing ground shoots. All thirty-two beakers were covered with Parafilm and allowed to soak for over 50 hours. The solutions were diluted by adding 10 ml of RO H_2O to the root solutions and 1 ml of RO H_2O to the shoot solutions. Each solution was then filtered using a 0.22 um syringe filter and covered with Parafilm for storage until analysis.

Analysis by FAAS

Flame Atomic Absorption Spectrometry (FAAS) was performed on each sample in order to determine lead concentration. Eleven standards were prepared by serial dilution with 0.1 M $Pb(NO_3)_2$ and distilled H_20, resulting in $[Pb^{2+}]$ of 0, 0.272, 0.544, 0.777, 1.04, 5.18, 10.4, 20.9, 29.8, 39.8, and 49.7 mg/L. A standard curve was constructed using these standards and their respective wavelengths detected by the PerkinElmer AAnalyst 200 (Figure 1). Lead concentrations within the experimental samples of digested plant material were then determined by comparing the absorbance value of each sample to the calibration curve created by the standards.

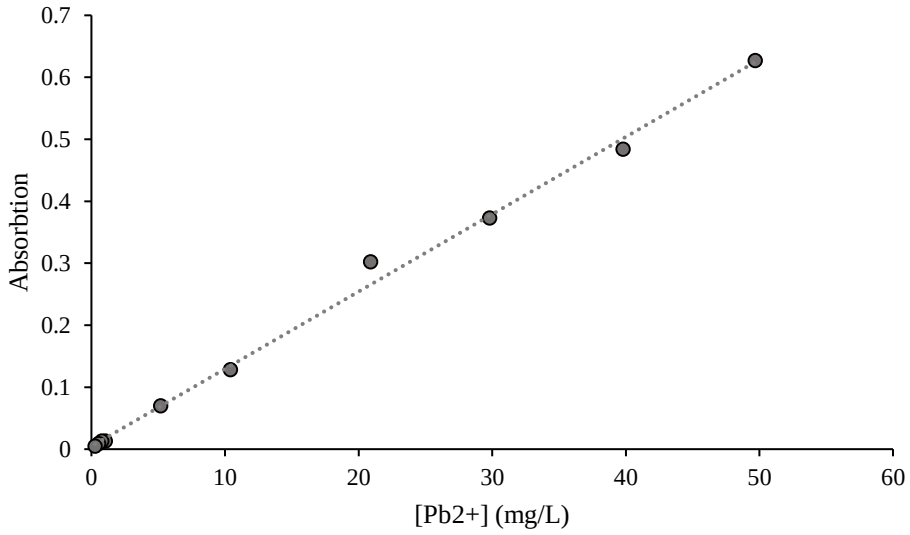

Figure 1. Standard curve created from solutions of known lead concentrations and absorption measured by FAAS. Equation of line was y = 0.0125x + 0.0047, where y represents absorbance and x represents lead concentration. R = 0.998.

Statistical Analysis

A Kruskal-Wallace test was performed to determine whether a significant difference existed between the amounts of lead absorbed in the roots and shoots of the *C. sativum* exposed to different amounts of lead. Statistical analysis was done using R. 3.3.3 and the package R Commander with alpha set to 0.05. Lead concentration absorbed in roots and shoots were analyzed separately. A *p* value less than 0.05 indicated a significant difference between the experimental groups. Additionally, an ANOVA was performed to determine whether a significant difference existed between the heights of the plants in the four treatment groups.

Results

Plants were watered with solutions containing different concentrations of lead over a period of 43 days. A solution of 10,000 mg/L Pb^{2+} was diluted to 1000 mg/L, 200 mg/L, 100 mg/L, or 50 mg/L (wt/vol) and used to water respective pots of *C. sativum*. Total lead exposure was calculated and recorded by deriving mass from cumulative volume of watering solution and lead concentration.

Lead concentrations were measured independently in the roots (Table 1 and Figure 2) and shoots (Table 2 and Figure 3) of each sample by FAAS. Absorption values were compared to a standard curve (Figure 1) to determine the amount of lead (wt/vol) present in each experimental sample. Average lead accumulations for 31 pots (four pots per treatment) were determined and graphed for both shoots and root samples (Figure 4). Values were standardized by dividing concentration of lead detected in each sample by mg of original plant material used in the nitric acid digest phase of the extraction to produce mg of Pb^{2+}/L of plant solution x mg of original plant material. An extreme outlier was removed from the roots data exposed to 110 mg of Pb^{2+}. Results of Pb^{2+} absorption did not meet parametric assumptions as it was not evenly distributed (Table 1), and therefore a nonparametric Kruskal-Wallace test was performed. As lead exposure increased, the amount of lead accumulated in the roots also increased significantly, $H(3) = 12.13$, $p = 0.007$; and in the shoots, $H(3) = 12.33$, $p = 0.006$. However, a significant difference only existed between the groups exposed to 55 mg, 110 mg, and 220 mg of lead, and the group exposed to 1100 mg of lead. Alpha was set to 0.05.

As an additional measure of plant growth during the exposure period, maximum heights of each plant were measured after 28 days of growth (Table 3). Data met parametric assumptions and an ANOVA test was performed. Plant height only differed significantly between the groups

exposed to 110 mg and 220 mg of lead, $F(3, 32) = 3.36$, $p = 0.031$. Germination rates were calculated at the four levels of lead contamination (Table 3). Lower rates of germination were observed when exposed to a higher amount of lead.

Table 1. Amount of lead (mg) absorbed in the roots of *C. sativum* over a 43-day exposure to lead-containing solutions, totaling 55 mg, 110 mg, 220 mg, and 1100 mg of lead. Values measured by FAAS and derived from a standard curve. An extreme outlier from the experimental group contaminated 110 mg was eliminated. Each sample represents a separate pot containing up to three plants, n = 15.

Pb^{2+} added to soil (mg)	**Standardized [Pb^{2+}] (mg/L*mg)**			
	Sample 1	Sample 2	Sample 3	Sample 4
55	0.011331	0.0079164	0.0211027	0.0218884
110	0.0254986	-	0.0343548	0.0393928
220	0.1191984	0.0345826	0.0258837	0.0290942
1100	0.2584001	0.3150329	0.1476845	0.1565508

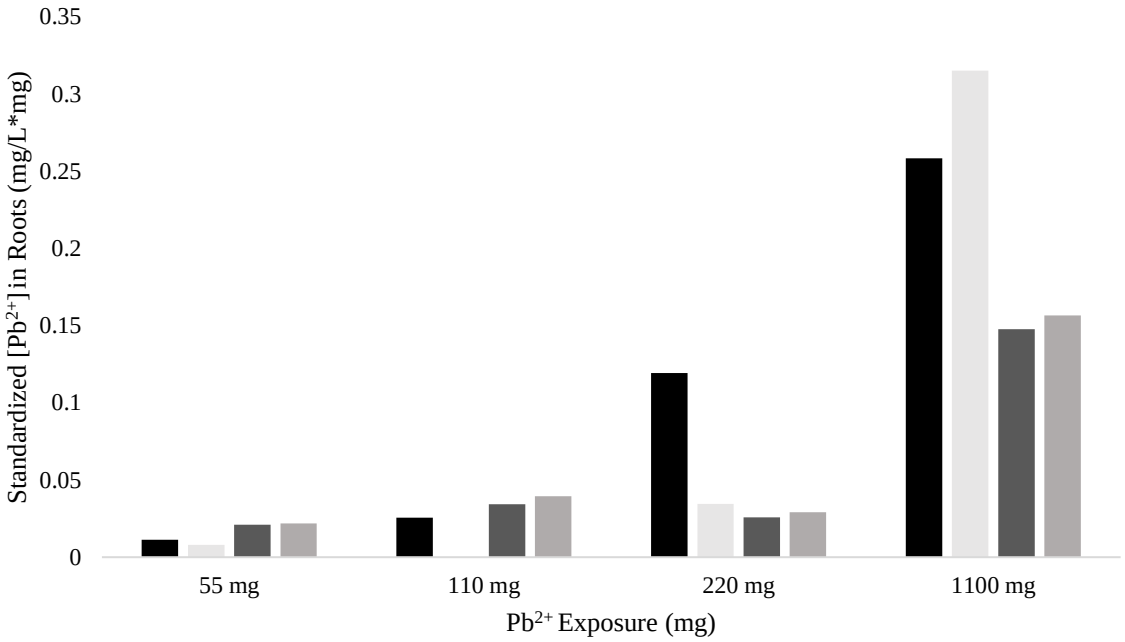

Figure 2. Amount of lead (mg) absorbed in the roots of *C. sativum* over a 43-day exposure to lead-containing solutions, totaling 55 mg, 110 mg, 220 mg, and 1100 mg of lead. Values measured by FAAS and derived from a standard curve. An extreme outlier from the experimental group contaminated 110 mg was eliminated. Each bar represents a separate pot containing up to three plants, n = 15.

Table 2. Amount of lead (in mg) absorbed in the shoots of *C. sativum* over a 43-day exposure to lead-containing solutions, totaling 55 mg, 110 mg, 220 mg, and 1100 mg of lead. Values measured by FAAS and derived from a standard curve. Each sample represents a separate pot, n = 16.

Pb^{2+} added to soil (mg)	**Standardized [Pb^{2+}] (mg/L*mg)**			
	Sample 1	Sample 2	Sample 3	Sample 4
55	0.0027077	0.0014051	0.0027264	0.0019482
110	0.0058932	0.0081761	0.0090079	0.0050694
220	0.0045	0.0126917	0.0088443	0.008187
1100	0.0392121	0.0119968	0.0294964	0.018197

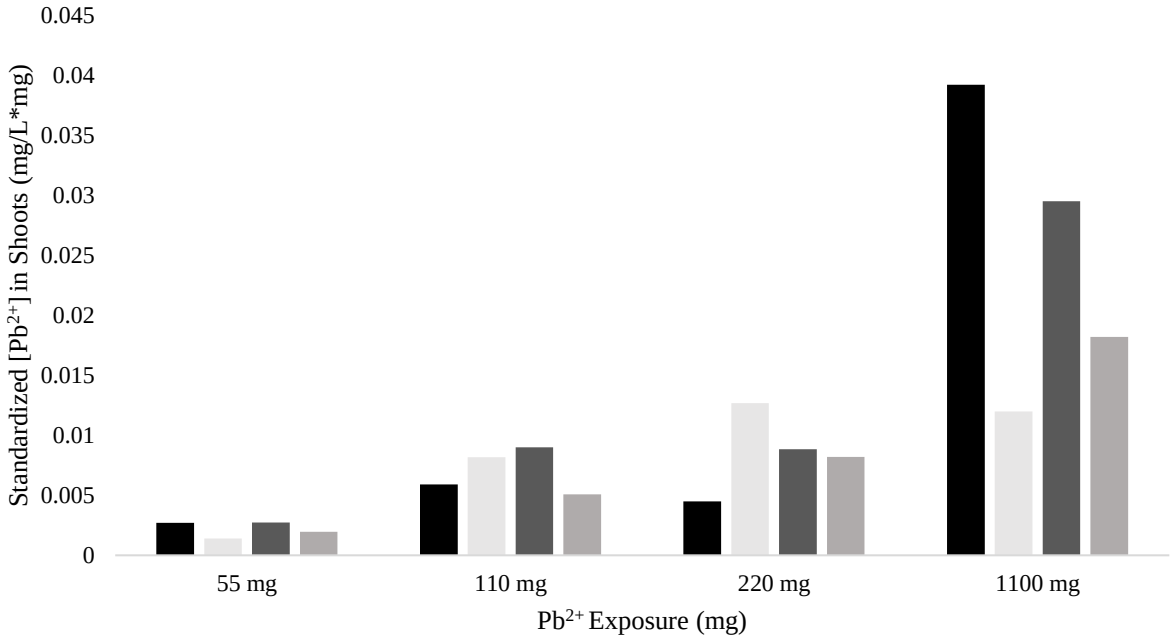

Figure 3. Amount of lead (in mg) absorbed in the shoots of *C. sativum* over a 43-day exposure to lead-containing solutions, totaling 55 mg, 110 mg, 220 mg, and 1100 mg of lead. Values measured by FAAS and derived from a standard curve. Each bar represents a separate pot, n = 16.

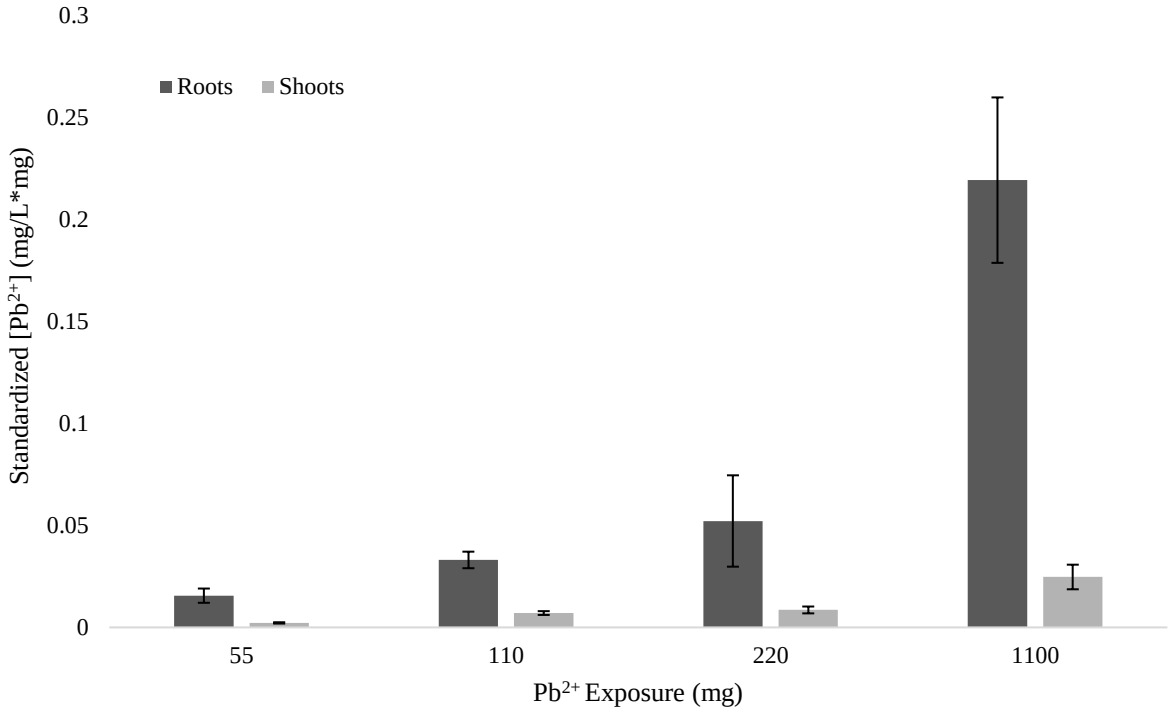

Figure 4. Average amounts of lead (mg) absorbed in the roots and shoots of *C. sativum* over a 43-day exposure to lead-containing solutions, totaling 55 mg, 110 mg, 220 mg, and 1100 mg of lead. Values measured by FAAS and derived from a standard curve. Each trial represents a separate pot, n = 31.

Table 3. Average heights and germination rate in each pot of *C. sativum* plants after 28-day exposure to lead-containing solutions, totaling 55 mg, 110 mg, 220 mg, and 1100 mg Pb^{2+}. There was a maximum potential of 12 plants per level of lead exposure, but only germinated seeds were considered in calculating average height.

Pb^{2+} added to soil (mg)	Average height (cm)	Germination rate
55	8.8	91.7%
110	10.1	83.3%
220	7.6	75.0%
1100	8.2	58.3%

Discussion

A positive correlation was shown to exist between the amount of lead to which *C. sativum* was exposed and the concentration of lead detected in the digested plant material, implying that *C. sativum* can absorb lead from the soil. However, due to certain details of our experimental design, it cannot be concluded that *C. sativum* would be economically useful as a phytoremediator. Phytoremediation of lead from soil can be a cost-effective method to decontaminate soils (Gupta, Huang, & Corpas, 2013); however, it is estimated to only be economically advantageous if the shoots accumulate at least 1% of the lead present in the soil (Huang et al., 1997). The scope and design of this experiment did not include a determination of the percentage of lead absorbed from the soil. Consequently, we were unable to determine whether or not *C. sativum* could be used as an economically advantageous phytoremediator. Future trials could record total plant weight and amount of lead in soil in order to calculate percentage of lead accumulated. This would allow us to directly evaluate the effectivity of *C. sativum* as a phytoremediator.

The decreased germination rate in plants exposed to a higher concentration of lead suggest that *C. sativum* may not be an ideal phytoremediator. However, other plants have shown similar reductions in germination as lead exposure increased. *Brassica pekinensis* was shown to absorb more lead depending on how much it was exposed to, but it also experienced a decrease in germination rate and root and shoot height with the increase in lead exposure (Xiong, 1997). Nevertheless, *B. pekinensis* was comparable to other known lead hyperaccumulators (Xiong, 1997). Because of its ability to hyperaccumulate, *B. pekinensis* can be used in phytoremediation (Rascio & Navari-Izzo, 2011). In this work, similar to *B. pekinensis*, *C. sativum* exhibited an increase in lead accumulation and a decrease in germination rate with increasing lead

contamination. Although *C. sativum* cannot be compared directly to hyperaccumulators such as *B. pekinensis* because percentage of lead accumulated was not calculated, it is possible that *C. sativum* may also be a hyperaccumulator and thus could participate in phytoremediation. However, future work should measure the percentage of lead *C. sativum* is able to accumulate in order to determine whether or not it is a hyperaccumulator.

There is the potential that *C. sativum* would not survive a significantly higher lead exposure. The EPA states that soil normally contains lead concentrations of 50 mg/L, but areas of concern such as cities can contain over 200 mg/L. The EPA does not allow a lead concentration over 400 mg/L in play areas, and 1200 mg/L in non-play areas ("Environmental Health and Medicine Education," 2007). While the concentration of lead tested would exceed the environmentally safe limit of lead concentration in play areas, it does not exceed the limit in non-play areas. To be useful in lowering concentrations in order to bring them below limits, *C. sativum* would need to be effective in absorbing lead when concentrations are over 1200 mg/L. Considering the decreased germination rate and health of *C. sativum* exposed to lead concentrations up to 1100 mg/L, higher concentration may pose a more serious threat.

Another question arising from this study is the mechanism of lead accumulation within *C. sativum*. Effective phytoremediators have demonstrated mechanisms of lead accumulation including phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization (Tangahu et al., 2011). The particular form of phytoremediation is dependent upon where in the plant heavy metals/toxins accumulate and how they are stored and/or broken down. Phytoextraction works by absorbing inorganic material from a plant's environment into its roots and shoots. Phytostabilization includes a variety of ways by which plants bind to and demobilize contaminants in the soil. Rhizofiltration is the absorption or precipitation of a substance into a

plant's roots. In phytovolatization, a plant absorbs a specific substance, which is then evaporated (Tangahu et al., 2011). Based on the higher detected lead concentration in the roots of *C. sativum*, it is possible that the primary mechanism of lead uptake involves either absorption within or fixation onto the surface of the roots. A small amount of lead was also absorbed into the shoots. Further research could focus on determining the specific localization pattern of lead accumulation within *C. sativum*. This experiment could be repeated using hydroponics instead of soil as the growth medium in order to eliminate the possibility of soil clinging to the outside of the plant. Additionally, plant tissue from various sections of the roots and shoots can be stained with sodium rhidizonate at a pH of 2.8 and analyzed microscopically. Lead causes sodium rhidizonate to form a scarlet precipitate, therefore allowing the location of lead to be detected (Glater & Hernandez, 1972). In addition, investigations into the biochemical mechanisms of storage and detoxification could be initiated. If the lead remains bound within the plant indefinitely, methods of proper disposal of lead contaminated plant material would have to be explored if *C. sativum* were to be used as a phytoremediator.

This study serves as a preliminary proof of concept study that can act as the foundation for further research in this area. A positive correlation was detected between *C. sativum*'s lead exposure and the concentration of lead bioaccumulated in its roots and shoots after 43 days, indicating the presence but not effectiveness of phytoremediation. *C. sativum* therefore maintains its strong potential of being effective in phytoremediation. However, more research should be done to confirm this potential, as well as to further test the vitality of the plant under stress from toxins, and to determine the method of hyperaccumulation if it is shown to exist. In future studies, a larger sample size should be used in order to increase statistical power.

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